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Microbiological diagnostics in periodontics: biological significance and clinical validity

ARIE J. VAN WINKELHOFF & EDWIN G. WINKEL

For over a century the medical profession has employed clinical microbiology as a tool in the diagnosis and treatment planning of infectious diseases. The identification of etiologic agents helps select the optimal drug therapy to support the patient in overcoming an infectious disease. Clinical microbiology in dentistry is used in cariology, implant dentistry, and periodontics. Recently, microbiology as a diagnostic tool in periodontics was evaluated, with the emphasis on sampling methods and different techniques to detect and quantify target bacteria (29). The present article discusses the rationale for applying clinical periodontal microbiology in the treatment of severe types of destructive periodontal disease. Microbiological analysis is useful when the information has the potential to direct clinicians towards more effective treatment strategies. The concepts presented here are based on the periodontal literature as well as experience in the field of clinical microbiology as it has existed in the Netherlands for over 15 years. The scientific literature will be used with the proviso that data can be interpreted in different ways and may sometimes result in opposite conclusions. In addition, the scientific basis for a rational use of clinical microbiology in periodontics is still incomplete. However, it is the opinion of these authors that the available information is sufficient to support the use of clinical microbiology in periodontal diagnosis and treatment planning. Since the use of microbial testing in periodontics is to a large, but not exclusive, extent related to the use of antibiotics, this paper will mostly focus on that relationship.

The discussion in this paper is based on the following views:

- periodontitis is a collection of etiologically different diseases;
- there is growing evidence that some periodontal pathogens have characteristics of exogenous microorganisms;
- microbiological testing can help select patients who are likely to benefit from systemic antimicrobial therapy;
- clinical microbiology in periodontics can contribute to cost-effective treatment.

Three basic steps in periodontics

The three main phases in periodontics include diagnosis, active anti-infective (cause-related) treatment, and restorative periodontal and dental procedures (53) (Fig. 1).

Diagnosis

Diagnosis assists in the selection of the most appropriate treatment based on individual treatment needs and takes into account individual risk factors such as smoking, stress, systemic diseases, and immunocompetence. Past and current medication for oral and nonoral disorders is checked. A detailed analysis of the dental status and the degree of periodontal destruction is also established. The patient's view on treatment needs completes the diagnostic phase. A microbial diagnosis in the diagnostic phase is sometimes desirable to assess the type and degree of therapeutic intervention.

Infection control

The anti-infectious approach in the active periodontal treatment phase consists of a number of measures to reduce the total bacterial load. Initial

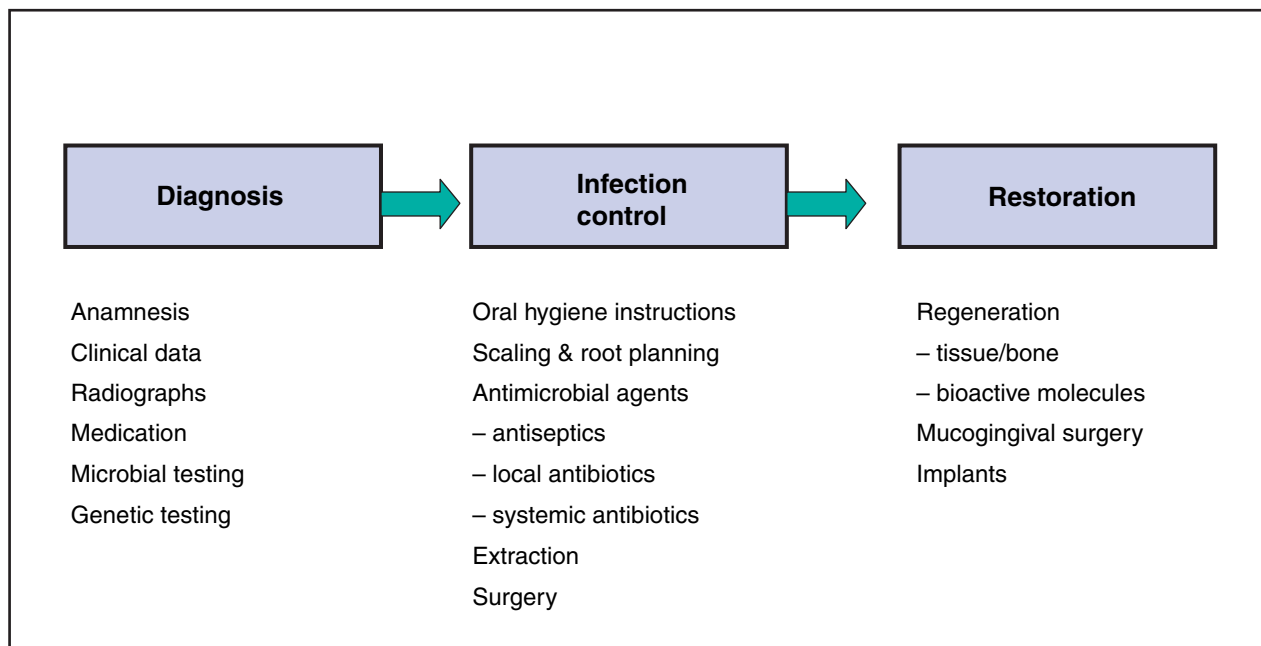


Fig. 1. Three basic steps in periodontics.

treatment comprises supra- and subgingival debridement and instruction in proper oral self-care. A systemic or local antimicrobial therapy may be initiated after completion of the initial periodontal treatment. To further reduce the subgingival bacterial load, periodontal surgery may be performed. At the end of the active periodontal treatment phase, the recall interval for periodontal maintenance is determined. A microbial analysis of the subgingival microflora at the end of the active treatment phase to test for remaining pathogens may help evaluate the efficacy of the anti-infectious measures and assist in determining recall frequency.

Restoration

Restoration is an important third treatment step in periodontics. In this phase, guided tissue or bone regeneration with barrier membranes may be used to restore part of the lost periodontal tissue. Biological active molecules to regain alveolar bone may be applied. To compensate for lost teeth, dental implants may be installed. It seems essential to perform restorative procedures only after periodontal health has been restored in the active treatment phase. Violating this rule may jeopardise planned restorative procedures due to recurrent infection. A microbiological analysis may guide the treatment of persistent infections prior to and after the completion of the restorative intervention (e.g. peri-implantitis, abscess formation).

Do we need microbiological information to treat infectious diseases?

In medicine, clinical microbiology is used for diagnosis and treatment planning.

Laboratory testing is only meaningful when the acquired information helps to direct treatment planning and when it assists in providing optimal therapy. However, the majority of medical infections are treated without any microbiological testing because the likelihood of having a known pathogen in a given type of infection is often high, and experience shows that a standard antimicrobial therapy is effective in the majority of such patients. For instance, since *Escherichia coli* causes approximately 85% of uncomplicated urine tract infection, and this pathogen is susceptible to a number of antibiotics, microbial testing is not a prerequisite to initiate antibiotic treatment. However, when the standard antibiotic regimen is clinically ineffective, in cases with infectious complications, or in recurrent disease, changes in treatment necessitate additional microbiological information. This approach to infectious disease management is used in relatively mild and uncomplicated medical infections, such as otitis media, upper respiratory infection, eye infections, etc.

In severe and potentially life-threatening infections or in infectious diseases with unpredictable etiology,

microbial testing is an essential part of the diagnosis. For instance, the clinical diagnosis of severe pulmonary infection is simple and relatively easy to obtain, but a number of viruses as well as *Mycobacterium tuberculosis*, *Legionella pneumophila*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, and various gram-positive bacteria including streptococci and staphylococci can cause the disease. It can be of critical importance to determine the etiology in specific cases of pneumonia in order to select the optimal antimicrobial therapy.

The bacterial component of periodontitis

The etiology and pathogenesis of periodontitis is much better understood now than a decade ago, and new risk factors are continually been identified. Several risk factors are very difficult (smoking, stress) or impossible to control (age, genetic traits).

Since bacteria cause gingivitis, periodontitis, and periodontal abscesses, periodontal treatment is aimed primarily at reducing the total periodontal bacterial load (supra- and subgingival plaque) and suppressing or eliminating certain target microorganisms from subgingival areas. Although gram-negative anaerobic rods and spirochetes dominate the subgingival microbiota of most periodontitis patients, it has become clear that marked qualitative and quantitative microbial differences exist among patients (14, 17, 21, 24, 38, 40, 47). Factors that influence the composition of the subgingival microbiota include age, poor oral hygiene, tobacco smoking, stress, systemic diseases, decreased immunocompetence (neutropenia), and genetic traits (20, 24, 32, 40, 47). The rate of periodontal disease progression is not necessarily determined by the same set of risk factors in each patient, and a relationship probably exists between different risk factors and various infectious agents. For example, in severe nonsmoker periodontitis patients, a relationship seems to exist between the carrier state of allele* 2 in both pro-inflammatory and the anti-inflammatory interleukin 1 genes, and the absence of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* (24). Despite these subjects suffering from severe periodontitis, they lacked all known risk factors (nonsmokers, no diabetes mellitus, no putative exogenous periodontal pathogens), and their subgingival microbiota consisted of commensal periodontal pathogens.

Do we need clinical microbiology in periodontics?

Microbiological testing is not necessary to diagnose the mere existence of gingivitis and periodontitis. Microbiological tests are not needed to confirm what a dental probe can easily reveal. Microbiological knowledge can, however, be helpful in the context of periodontal therapy. The primary approach to the treatment of periodontitis is mechanical debridement with or without periodontal surgery. Basic periodontal treatment has been shown to be successful in arresting the disease activity in the majority of adult patients with chronic periodontitis (6), especially when combined with good maintenance care (3, 4). A poor treatment response, sometimes referred to as refractory periodontitis, may occur in patients with aggressive periodontitis. The prevalence of refractory periodontitis is difficult to determine because different definitions of the condition are used and many different treatment protocols are applied. Disease activity can recur in maintenance patients. For some types of periodontitis, such as juvenile periodontitis, the disease may recur in up to 25% of patients within 1 year following active therapy (25). *A. actinomycetemcomitans* has been identified as a microbial risk factor for poor treatment response, and a persistence of the organism is associated with disease recurrence in localized juvenile periodontitis (15). A poor treatment response in adults with periodontitis has also been related to a number of bacterial species. For instance, the persistence of *A. actinomycetemcomitans*, *P. gingivalis* and *Tannerella forsythia* (formerly *Bacteroides forsythus*) after mechanical periodontal debridement has been associated with only moderate improvement in gingival bleeding on probing, probing pocket depth, and clinical attachment level (39, 45, 59) and with further loss of alveolar bone height (14). Ongoing periodontal attachment loss in maintenance patients has been related to the persistence of, among others, *P. gingivalis*, *Prevotella intermedia*, and *A. actinomycetemcomitans* (16, 56). Available evidence thus shows that certain microbial complexes are associated with a poor treatment response and ongoing or recurrent periodontal disease activity in susceptible patients. Refractory periodontitis patients may benefit from microbiological testing to identify the presence and levels of bacteria that could be a target for further treatment, especially when considering adjunctive systemic antibiotic therapy.

Endogenous vs. exogenous periodontal pathogens

Most cultivable subgingival bacterial species in periodontitis are part of the normal oral microflora. Common oral bacterial species accumulate in the subgingival area and, over time, may constitute a significant part of subgingival plaque. Periodontitis associated with commensal periodontal bacteria may be considered a commensal or opportunistic infection. It is questionable whether *A. actinomycetemcomitans* and *P. gingivalis* are part of the normal periodontal microflora (for review see [44]). Both species have the characteristics of exogenous pathogens rather than of opportunistic pathogens (18). This presumption is partly based on the finding that both species exhibit a low occurrence in periodontally healthy subjects, related to Koch's first postulate of causality. The concept of exogenous periodontal bacteria has gained strength as a result of recent studies. Griffen et al. (19) used a sensitive polymerase chain reaction technique to detect *P. gingivalis* in adult patients with periodontitis and in periodontally healthy subjects. They concluded that, based on the

low prevalence in periodontal health, *P. gingivalis* is not a member of the normal periodontal microflora. In a similar study, using anaerobic culture techniques, van Winkelhoff et al. (50) confirmed the low occurrence of *P. gingivalis* in periodontally healthy subjects (10.6%) and found a strong association of this pathogen with destructive periodontal disease (OR = 12.3). *A. actinomycetemcomitans* was detected in only 12.8% of the subjects without periodontitis (50). Boutaga et al. (9) applied a sensitive real-time polymerase chain reaction technique to detect *P. gingivalis* and *A. actinomycetemcomitans* in periodontal health and disease and found the prevalence of *P. gingivalis* and *A. actinomycetemcomitans* in periodontal health to be only 10% and 18%, respectively. The subgingival prevalence of *A. actinomycetemcomitans* and *P. gingivalis* seems related to age and treatment history, as *A. actinomycetemcomitans* tends to decrease and *P. gingivalis* to increase with increasing age in periodontitis patients (40). Indeed, *A. actinomycetemcomitans* and *P. gingivalis* may be considered true infectious agents in the human oral cavity (Fig. 2). This concept has clinical implications as it allows for a differentiated treatment

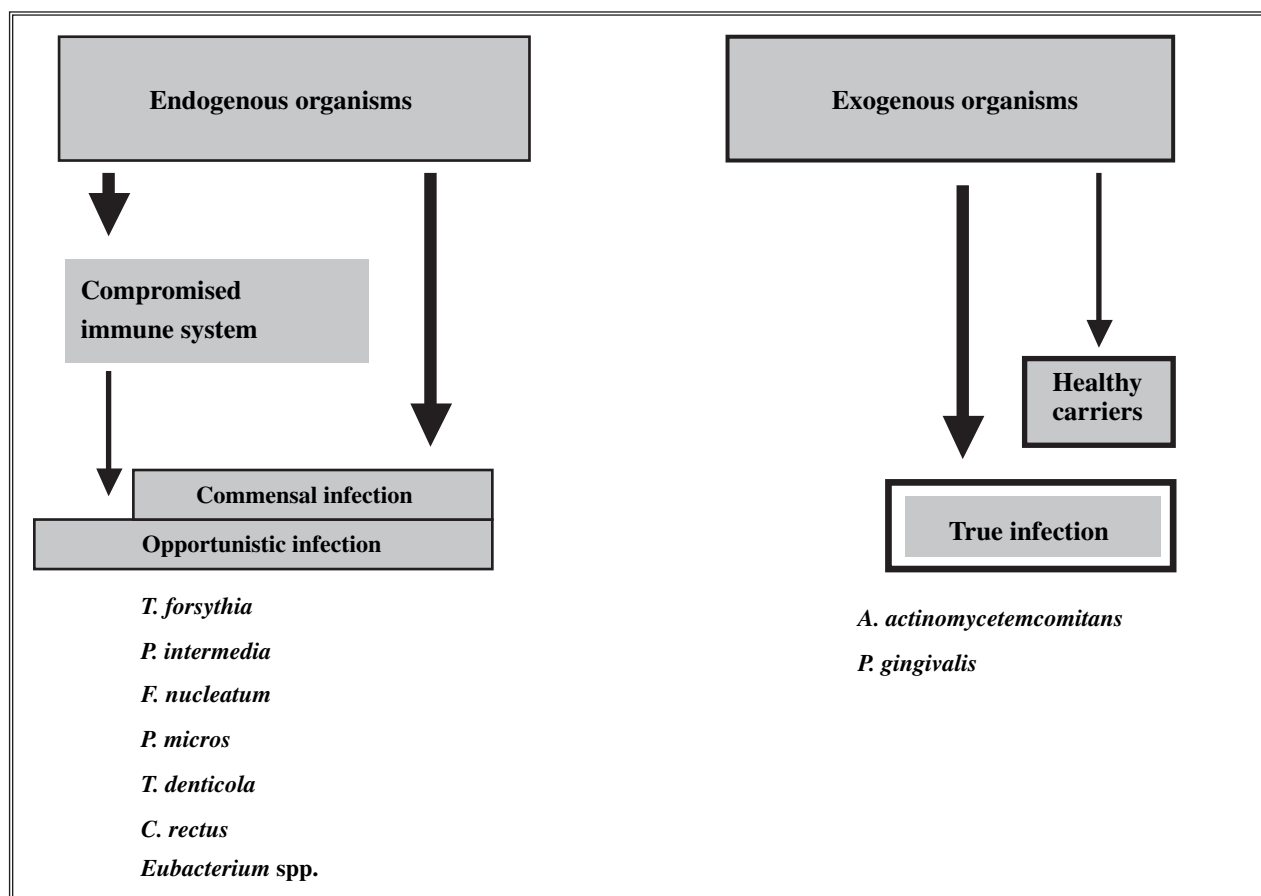


Fig. 2. Different periodontal infections on the basis of the origin of the pathogens.

strategy: endogenous pathogens may merely need to be reduced in subgingival sites, whereas exogenous pathogens usually should and can be eliminated from infected subjects (51). It should also be noted that data on the prevalence of periodontal pathogenic bacteria are based mainly on studies of western populations and may be not pertain to different ethnic groups (41, 46). So far, however, there are no data to show that pathogens behave differently in different ethnic populations (45). In an Indonesian population, Timmerman et al. (46) showed that *A. actinomycetemcomitans* is a periodontal disease susceptibility factor and that *P. gingivalis* is associated with periodontal disease progression.

Relationship between microorganisms and the outcome of periodontal treatment

A. actinomycetemcomitans and *P. gingivalis* cannot be removed from a significant part of deep periodontal lesions by mechanical therapy alone (for review see [44]), as demonstrated in localized juvenile periodontitis (15, 52) and in adult periodontitis patients (39, 40, 59). The percentage of *A. actinomycetemcomitans* and *P. gingivalis* may even increase following scaling and root planing (33, 39). Chaves et al. (14) showed that periodontal lesions with detectable *P. gingivalis* at 1, 3 and 6 months after debridement showed further alveolar bone loss,

whereas lesions without this pathogen at any time point post-treatment tended to show alveolar bone gain (Fig. 3). The subgingival persistence of *T. forsythia* has also been associated only with a moderate improvement in clinical periodontal status (45, 59). Incomplete removal of certain subgingival pathogens is related to initial probing pocket depth (33, 35), the location of the lesion, the anatomy of the tooth (30), compliance with instructions for oral home care (32), and possibly the immunocompetence of the patient (20).

Are specific pathogens predictors of further periodontal attachment loss?

There are sufficient data to support the important role of specific periodontal bacteria in progressive periodontitis of treated patients. In one study, adult patients with refractory periodontitis were investigated clinically and microbiologically at baseline and at 12 months' post-treatment (56). Periodontal lesions without detectable *A. actinomycetemcomitans*, *P. gingivalis*, or *P. intermedia* showed no further clinical attachment loss, whereas 20% of sites with detectable levels of one or more of these pathogens experienced ≥ 2 mm additional attachment loss (56). In a 5-year follow-up study, only periodontal pockets without detectable *A. actinomycetemcomitans* or *P. gingivalis* and $< 5\%$ *P. intermedia*

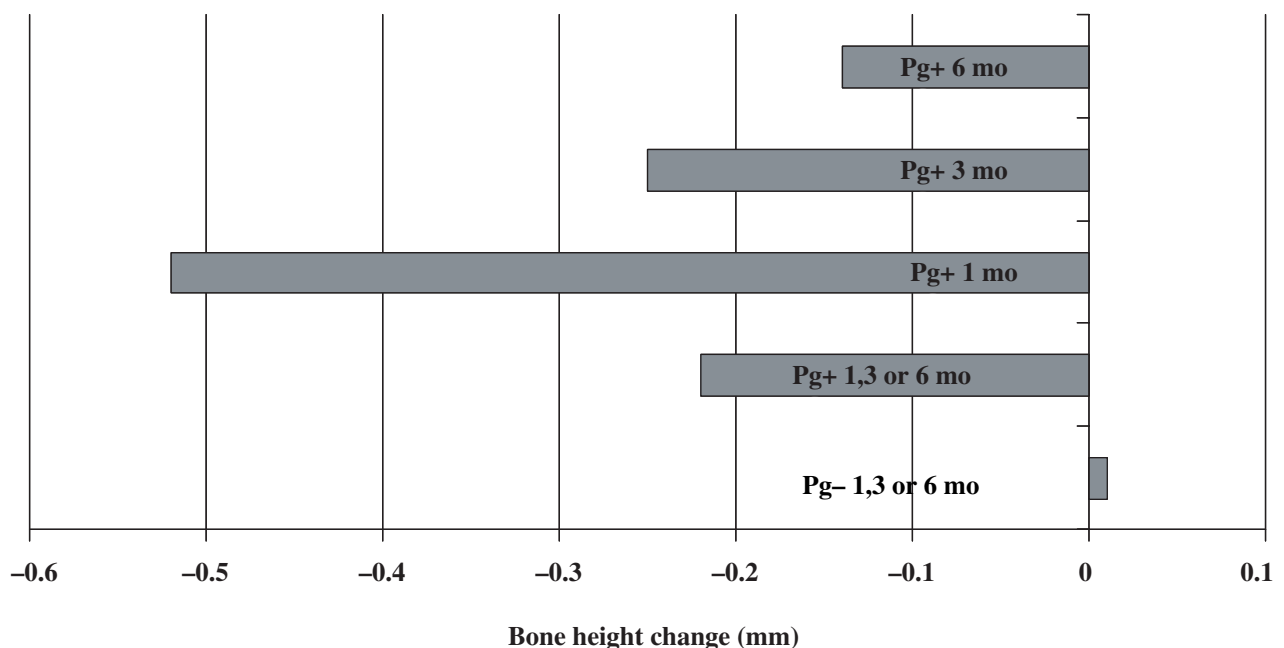


Fig. 3. Presence of *P. gingivalis* (Pg) and bone height change 1, 3, and 6 months after initial periodontal treatment (data from [14]).

remained stable, whereas 67% of sites testing positive for one or more of these species lost attachment (16). Longitudinal and retrospective studies from various laboratories have also indicated an increased risk for periodontal breakdown in sites positive for specific periodontal pathogens (10, 12, 21, 38, 42, 43). According to Machtei et al. (31), periodontitis patients with detectable *T. forsythia* at baseline had more deteriorating sites and twice as much tooth mortality as patients without this pathogen. Risk factors for incomplete removal of periodontal pathogens may be smoking, poor oral hygiene level, and insufficient scaling and root planing. One criterion for the end-point of periodontal treatment is suppression of certain bacterial pathogens to below the level of detection. Clinical periodontal microbiology can play a role in designing effective therapies and in monitoring treatment efficacy (2).

Systemic antibiotics

Antibiotics can kill or inactivate bacteria that are inaccessible to periodontal instrumentation, they can enhance the effects of mechanical periodontal treatment, they can reduce the risk for refractory or recurrent disease, and they can reduce the number of teeth that need periodontal surgery. One strategy of antibiotics in periodontics is to use these drugs on the basis of clinical need. This approach is sometimes employed for severe periodontitis at a young age or for certain clinical conditions such as angular bony defects and suppuration. Other reasons for considering systemic antimicrobial treatment may be poor treatment response after initial periodontal treatment, expressed as only a moderate pocket reduction, little or no attachment gain, or a high percentage of residual bleeding sites despite good oral hygiene compliance.

There is a body of evidence suggesting that, when used as adjuncts to mechanical treatment, systemic antibiotics can significantly improve periodontal treatment outcome (8, 26, 44, 51, 57, 58). Members of the 4th European Workshop on Periodontology produced a systemic review on the effects of systemic antibiotic therapies as adjuncts to mechanical periodontal treatment in periodontitis patients. Meta-analyses revealed that treatment with spiramycin, metronidazole and metronidazole plus amoxicillin significantly improved the effect of scaling and root planing compared to controls and placebo-treated patients (22). However, those studies did not use the microbial composition of the subgingival plaque as a

selection criterion for antibiotic therapy, and the choice of antimicrobial therapy was empiric and based on the experience of the clinician. Such approach does not consider the possibility that some pathogens may display resistance to the tested drugs. This may occur for *A. actinomycetemcomitans*, which is not susceptible to metronidazole when used as a mono-therapy. Therefore, clinical studies carried out without selecting antibiotics on the basis of microbial variables may underestimate the potential effectiveness of periodontal systemic antibiotic therapy.

A more targeted approach to periodontal antibiotic therapy is microbiological analysis of the subgingival microflora to determine the presence and levels of periodontal pathogens. The decision whether or not to prescribe a systemic antimicrobial treatment and subsequently the choice of an antibiotic is then based on the microbiological outcome of the analysis (2). This approach has several advantages.

Culturing of subgingival key pathogens opens the possibility of antibiotic susceptibility testing that can provide information on the most optimal antibiotic choice and regimen. The selected antimicrobial therapy can be based on known susceptibility profiles of the target microorganisms and on documented effectiveness in the periodontal literature. For example, clindamycin generally is not effective against *A. actinomycetemcomitans* and consequently will fail to eradicate or significantly suppress this pathogen in infected periodontal sites. Patients with high levels of beta-lactamase producing bacteria may not benefit from unprotected amoxicillin therapy (23, 53).

Patients who are unlikely to benefit from systemic antimicrobial therapy can be excluded. Periodontitis patients testing positive for *P. gingivalis* and *A. actinomycetemcomitans* at baseline seem to be prime candidates for adjunctive systemic antibiotic treatment. Flemmig et al. (17) showed that systemic metronidazole plus amoxicillin treatment following scaling and root planing was only effective in *A. actinomycetemcomitans*-infected patients. In the absence of *A. actinomycetemcomitans*, adjunctive antibiotic therapy showed no clinical benefit over scaling and root planing alone. This important observation indicates that microbiological selection of patients positive for *A. actinomycetemcomitans* is clinically relevant and overtreatment with antibiotics can be prevented by excluding subjects without this pathogen. Winkel et al. (57) used a double-blind placebo-controlled randomized protocol to investigate the clinical and microbiological effects of metronidazole plus amoxicillin. In that study, patients

were not selected on the basis of microbiological parameters, i.e. microbial analysis of the subgingival plaque was also performed blindly (57). Probing pocket depth reduction, clinical attachment gain, and reduced bleeding on probing occurred more often in patients receiving antibiotic medication than in placebo-treated subjects. Further analysis showed that the additional clinical effects of the antibiotics in terms of probing pocket depth reduction (Fig. 4) and reduction in the number of pockets ≥ 5 mm (Fig. 5) could be attributed to patients who were culture-positive for *P. gingivalis* at baseline. *P. gingivalis*-negative subjects at baseline treated with metronidazole plus amoxicillin showed no significant improvements in pocket depth and in number of sites with probing depth ≥ 5 mm compared to placebo-treated subjects. These observations are evidence that antibiotic treatment as an adjunct to mechanical debridement benefits a select group of periodontitis patients, and support the notion that microbiological testing prior to antibiotic therapy is a rational diagnostic approach in periodontics.

The over- and misuse of antibiotics can be reduced, thereby not contributing to the increasing problem of

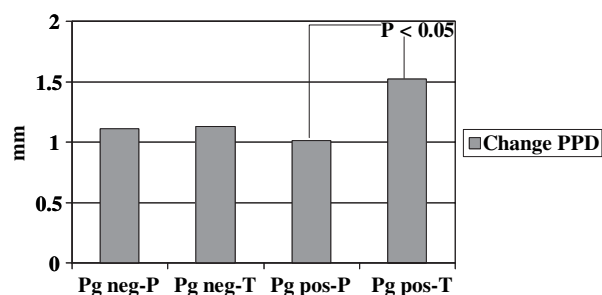


Fig. 4. Change in full-mouth pocket probing depth (PPD) in *P. gingivalis* (Pg)-infected and noninfected patients at baseline after metronidazole plus amoxicillin (T) or placebo (P) medication (data from [57]).

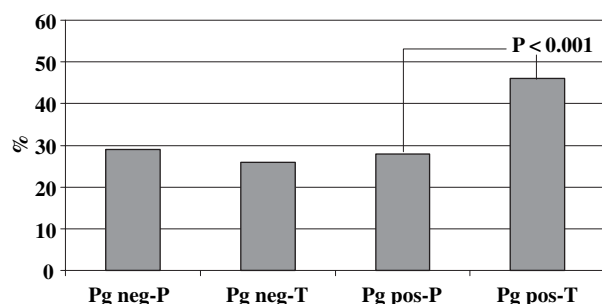


Fig. 5. Mean reduction in percent sites with probing pocket depth ≥ 5 mm in *P. gingivalis* (Pg)-infected and noninfected patients at baseline after metronidazole plus amoxicillin (T) or placebo (P) medication (data from [57]).

antimicrobial resistance. Apart from clinical and microbiological arguments for the selective use of systemic antimicrobial therapy in periodontics, there is the global problem of emerging antimicrobial resistance among human pathogens. Uncontrolled use, misuse and poor compliance are major causes of the growing phenomenon of microbial resistance, which poses a serious challenge in the control of infectious diseases and threatens public health in developed and underdeveloped countries. For instance, antimicrobial resistance of *Streptococcus pneumoniae* to penicillin is correlated with the local use of beta-lactam antibiotics and macrolides (11). Emerging antimicrobial resistance has also been noted for periodontal bacteria. The level of resistant periodontal bacteria towards beta-lactam antibiotics, metronidazole, clindamycin, and tetracycline was found to be significantly higher in periodontitis patients in Spain than in the Netherlands (49). This finding could be related to a significant higher intake of antibiotics in Spanish patients. In a study of antibiotic use in European countries, Cars et al. (13) found a great variation in antibiotic consumption among various countries. The use of antibiotics was significantly higher in Mediterranean countries than in other European countries. The Netherlands was the country with the lowest antibiotic use and France and Spain the countries with the highest (Fig. 6). Restricted and controlled use and improved compliance seem to be the best strategies to overcome the problem of antimicrobial resistance. Therefore, to limit medication to patients most likely to benefit from the therapy, prescription of antibiotics in periodontal patients should be based on defined clinical and microbiological parameters rather than on an empiric approach to prescription. Also, the institution of antibiotic regimens without scientifically demonstrated clinical effects should be avoided.

Selection of effective antibiotics at the time of initial therapy will contribute to a cost-effective treatment. Microbiological testing prior to systemic antimicrobial treatment will prevent unnecessary use of antibiotics, thereby reducing the costs of medication and also of the overall treatment (17, 57). There is evidence that optimal use of antibiotics reduces the total number of teeth in need of surgical intervention (28, 57). In contrast, repeated root debridement in patients with ongoing disease activity is time-consuming and costly, and is often ineffective (5). In addition, there are potentially unwarranted effects of repeated periodontal mechanical treatment, such as loss of tooth substance and recession of gingiva in shallow sites.

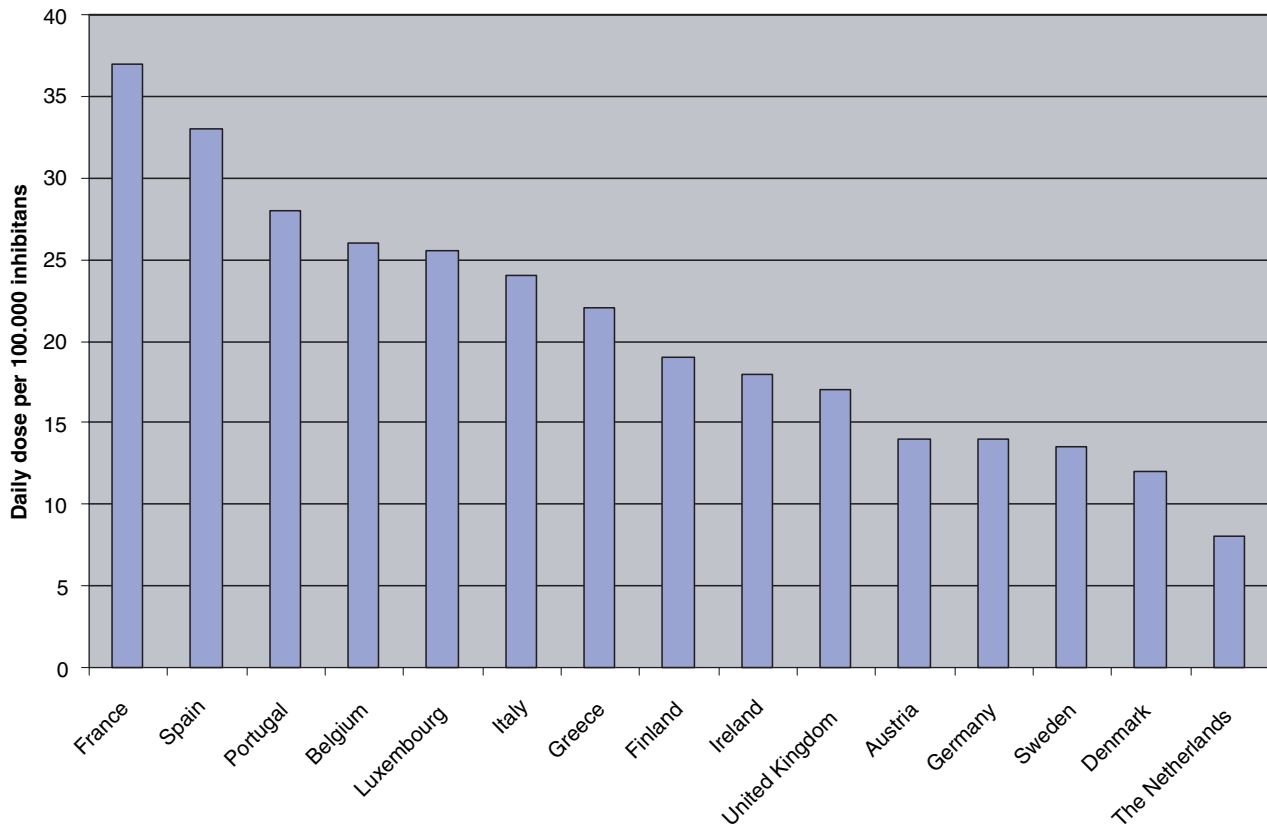


Fig. 6. Daily dose of antibiotics per 100,000 inhabitants in different countries of the European Union (data from [13]).

Practical aspects

Microbiological sampling

The topic of microbiological sampling has been discussed recently (29). A practical and economic approach to sample major pathogens from the sub-gingival area is to pool samples from the deepest bleeding pockets of molar teeth in each quadrant (34). A sampling strategy based on site-specific information is of less importance as a precursor for systemic antibiotic therapy.

Types of microbial tests

The culture technique represents an open test system and allows the detection and quantification of all cultivable bacterial species. It also enables the detection of unusual pathogens and superinfecting organisms, such as *Candida* species, staphylococci, enterococci and other enteric organisms, which may occur in medically compromised patients and after unsuccessful systemic periodontal antibiotic therapy. Antibiotic susceptibility testing can only be applied after cultivation and isolation of target species. Polymerase chain reaction techniques have been

developed to detect periodontal pathogens and results of these tests are in agreement with culture results (9).

Microbial testing

Testing before active periodontal treatment. Patients suffering from aggressive periodontitis run the risk of continuing periodontal disease activity after mechanical depuration and can benefit from microbial testing prior to initial treatment. Most aggressive periodontitis patients show significantly improved healing after systemic antibiotic treatment.

Control testing after active treatment. Because a marked suppression of periodontal pathogens is associated with periodontal stability (8, 14, 16, 37, 56), microbial testing can assist in determining the endpoint of periodontal treatment and in establishing the length of the recall interval. Control microbiological testing may especially be opportune in patients scheduled for implant dentistry to assure that major periodontal pathogens have been eradicated and commensal bacteria have been sufficiently suppressed prior to implant placement (48).

Refractory periodontitis. Subjects with refractory periodontitis are a group of patients who can benefit from microbial testing and subsequent antibiotic therapy. The diagnosis of refractory periodontitis should be reserved for patients who experience little reduction in bleeding on probing and pocket depth despite diligent mechanical debridement and excellent oral hygiene. Continuing suppuration may also constitute a sign of ongoing active disease.

Maintenance patients. The aim of regular maintenance therapy is to interfere with the recolonization of subgingival sites by potentially pathogenic bacteria. Patients with recurrent disease activity in the maintenance phase may benefit from re-treatment by scaling and root planing, reinforcement of oral hygiene measures, periodontal surgery or local application of an antimicrobial agent. Microbial testing in these cases can be performed as needed. Patients with recurrent periodontal disease associated with *A. actinomycetemcomitans* and/or

P. gingivalis or high levels of *T. forsythia* may benefit from antibiotic treatment.

Figure 7 presents a practical approach to microbial testing and the use of antibiotics in patients with severe types of periodontitis.

Microbiological considerations for sequencing of antibiotics

Antimicrobial agents can be used at different times in the active treatment phase. A large number of clinical studies have employed antibiotics during the initial treatment phase. However, there are microbiological arguments that speak for the use of systemic antibiotics after the completion of initial treatment.

- Antibiotics work best when the supragingival plaque level is low. Low plaque levels are typically found after rather than before or during initial periodontal treatment.

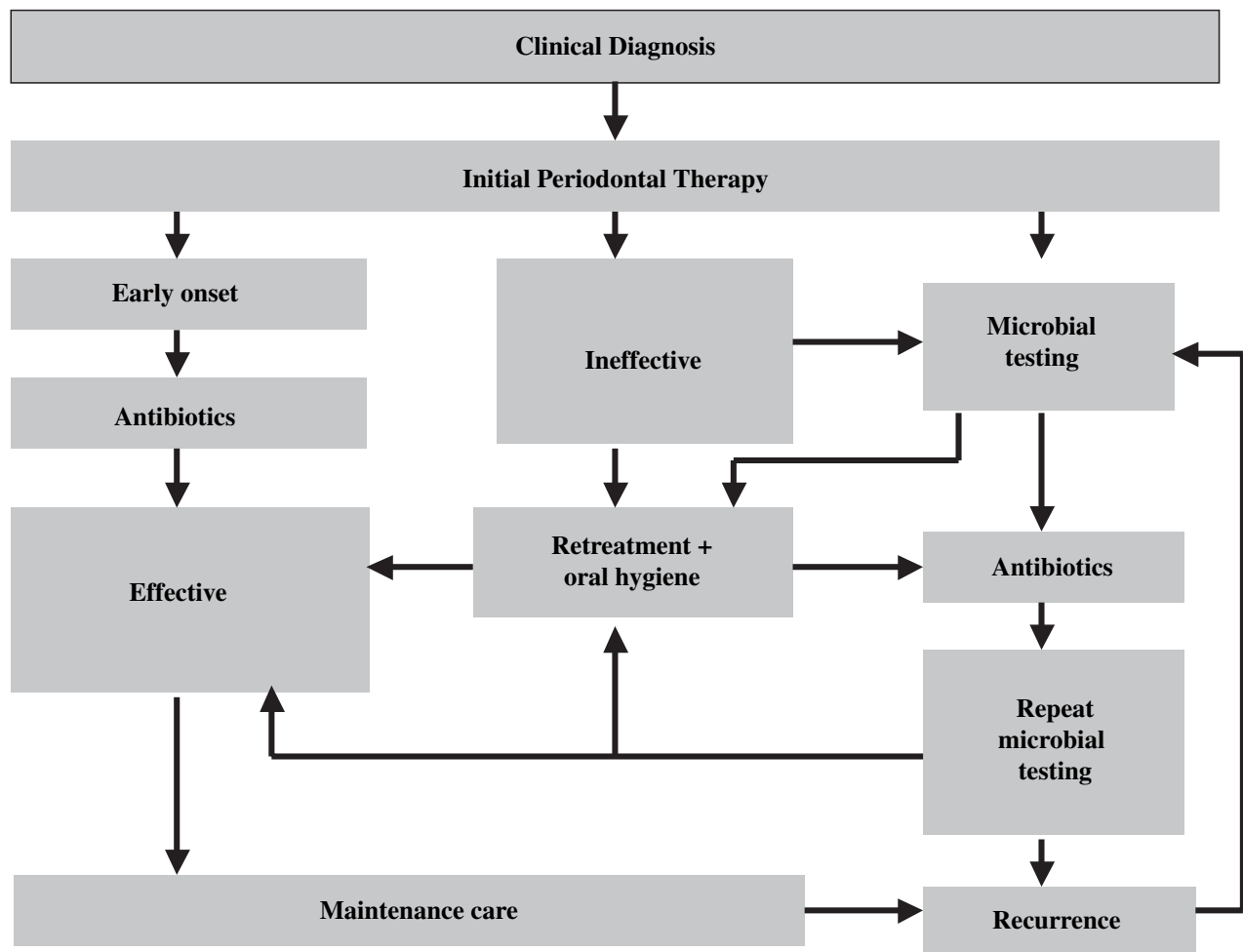


Fig. 7. Flow chart of microbial testing and the use of antibiotics.

- Antibiotics work best when the subgingival bacterial load has been significantly reduced by mechanical debridement (inoculum effect). In particular, a reduction of the commensal (endogenous) microflora should be attempted.
- Mechanical treatment can disrupt the bacterial biofilm on the root surface, which may increase the effectiveness of antimicrobial agents against resident bacteria. Loesche et al. (27) demonstrated that systemic metronidazole therapy is clinically more effective when delivered after mechanical debridement than during the mechanical treatment phase. Use of potent antibiotics without thorough mechanical debridement and without proper oral hygiene measures should be regarded as improper (2).
- To reduce the oral load of periodontal pathogens, teeth with a poor prognosis may be extracted prior to an antibiotic therapy.

Antimicrobial regimens

Microbial analysis of the subgingival microflora in periodontitis allows a differentiated choice of systemic antibiotics. Table 1 lists antibiotic regimens against some marker bacteria. Several antibiotics have predictable effects on target organisms. Metronidazole is effective against *P. gingivalis*, *P. inter-*

media, and most other gram-negative anaerobic rods. Amoxicillin acts against a wide range of subgingival bacteria but may, unprotected, be inactivated by beta-lactamases (23, 54). Clindamycin and tetracyclines also have broad spectra of activity on the subgingival microflora. Predictable suppression of subgingival *A. actinomycetemcomitans* requires metronidazole plus amoxicillin or other combinations of antibiotics (51).

It should also be noted that antibiotic regimens used in many clinical trials are not necessarily those that will display the optimal effect in daily practice. Based on $20 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ body weight, a daily dose of 750 mg of metronidazole can only treat an adolescent of 40 kg. In adults, a dose of $1500 \text{ mg} \cdot \text{day}^{-1}$ is indicated (55).

Parameters that determine the dosage of an antimicrobial agent include:

- susceptibility of the pathogen(s);
- severity of the infection;
- body mass (standard dose should adjusted for under- and overweight patients);
- other medications.

Smokers with periodontitis may benefit from prolonged medication time since smoking decreases the gingival blood flow and the amount of crevicular fluid, and thereby the exposure of subgingival pathogens to systemic antibiotics (36).

Table 1. Antibiotic regimens against marker bacteria

Indication	Antimicrobial therapy	Usual dosage	Reference
<i>P. gingivalis</i>	Metronidazole	250–500 mg tid 7–10 days	27, 28, 58
<i>T. forsythia</i>			
<i>Treponema</i> spp.			
Gram-negative anaerobes, absence of <i>A. actinomycetemcomitans</i>	Clindamycin	300 mg qid 7–8 days	22
Nonspecific infection	Doxycycline Spiramycin	100–200 mg 1 × day 7–14 days 1.0 g bid 7 days	1, 7
<i>A. actinomycetemcomitans</i> or <i>P. gingivalis</i> with high numbers of gram-positive pathogens	Metronidazole + amoxicillin	250–500 mg tid, 375–500 mg tid, both 7 days	8, 17, 51, 52, 57, 59
<i>A. actinomycetemcomitans</i> , hypersensitivity towards amoxicillin	Metronidazole + cefuroximaxetil	250–500 mg tid 250–500 bid, both 7 days	53
<i>A. actinomycetemcomitans</i> , hypersensitivity towards beta-lactams, presence of susceptible enteric microorganisms	Metronidazole + Ciprofloxacin	250–500 mg tid 500 mg bid, both 7 days	53

Conclusions

Microbial analysis in periodontics aims to:

- discriminate between different microbial types of periodontal infections;
- select subjects likely to benefit from adjunct systemic antimicrobial therapy;
- assist in selecting the most appropriate antibiotic treatment in accordance with the composition of the subgingival microflora;
- contribute to minimizing overuse of potent antimicrobial agents and the emergence of antimicrobial resistance;
- screen for horizontal and vertical transmission of periodontal pathogens among family members;
- help to determine the endpoint of active periodontal treatment and to establish the recall interval for periodontal maintenance care;
- help select patients in need for periodontal treatment before inserting implants in partially edentulous subjects. This may especially be indicated in subjects with a history of periodontitis (48).

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